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Geographical distribution of *Austrolebias monstrosus* (Huber, 1995), *A. elongatus* (Steindachner, 1881) and *A. vandenbergi* (Huber, 1995) (Teleostei: Cyprinodontiformes), with comments on the biogeography and ecology of Rivulidae in Pampasic and Chaco floodplains

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Abstract: We present a new record for *Austrolebias elongatus* from Gualeguaychú, in Entre Ríos province, Argentina, based on new fieldwork and a revision of material deposited in national ichthyological collections. We also give evidence on the erroneous records of *Austrolebias monstrosus* and *A. vandenbergi* from Ituzaingó, Corrientes province, as well as present additional records from Salta province for those species. Material previously determined as *A. elongatus* from Santiago del Estero is attributed to *A. monstrosus*. We restrict the distribution of these two species to Semi-arid Chaco Region in Argentina, Paraguay and Bolivia.

Key words: killifish; *Austrolebias accorsii*; annualism; Serafin Pierotti, Pampa

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Austrolebias Costa, 1998 is a genus of seasonal killifish of the family Rivulidae with 43 valid species inhabiting the La Plata basin and the Laguna dos Patos-Mirim system, with one recently described species from the upper Mamoré basin in the Amazon system (Nielsen and Pillet 2015). *Austrolebias elongatus* (Steindachner, 1881) was described, as *Cynolebias elongatus,* from specimens from the La Plata basin (by Steindachner 1881). Here we present a new record of this species based on additional specimens collected by Elizalde during the 1940s at Gualeguaychú, Entre Ríos province (Figure 1). This record represents the first from this province and the first from the right bank of the Uruguay River.

Austrolebias monstrosus Huber, 1995 and *Austrolebias vandenbergi* Huber, 1995 were described from specimens that came from the dry Chaco of Paraguay (Huber 1995), *A. monstrosus* from Boquerón province, near La Serena, 21.94° S, 059.97° W (Costa [2006] mistakenly gave the latitude as "56.97° W"), and *A. vandenbergi* from Presidente Hayes province, near Fortín Toledo. Later, Braga (1999) reported these species in Argentina from Laguna Yema, Formosa province (localities not included in Costa [2006]). In a subsequent revision of the genus, Costa (2006) restricted the distribution of these species to "Río Paraguay-Paraná basin in Paraguay and northern Argentina". Included was a record for both species from Ituzaingó, Corrientes province, Argentina, and also a record for *A. monstrosus* listed under Corrientes province as "MCNI 1142b, 1; Ruta Provincial 52, General de San Martin; G. Gonzo and M. Fabrezi, 13 Apr. 2005" (Costa 2006), those records represent the only known records of those species in the Paraná-Paraguay basins actually. We confirmed that this last locality is actually in Salta province, northwestern Argentina (G. Gonzo pers. com.). We retraced the Pierotti and Budin's expedition of the year 1950 and conclude that the records of *A. monstrosus* and *A. vandenbergi* from Ituzaingó, Corrientes, are mistaken.

We add new localities from Salta and the Chaco for *A. vandenbergi* and *A. monstrosus* and include the records of *A. monstrosus* (Osinaga, 2006) and *A. vandenbergi* (Montaña et al. 2012) from Bolivia (Figure 1).

The ichthyological material in the collections of Museo Argentino de Ciencias Naturales (MACN-ict), Museo de La Plata (MLP), Fundación Miguel Lillo (CI-FML) and

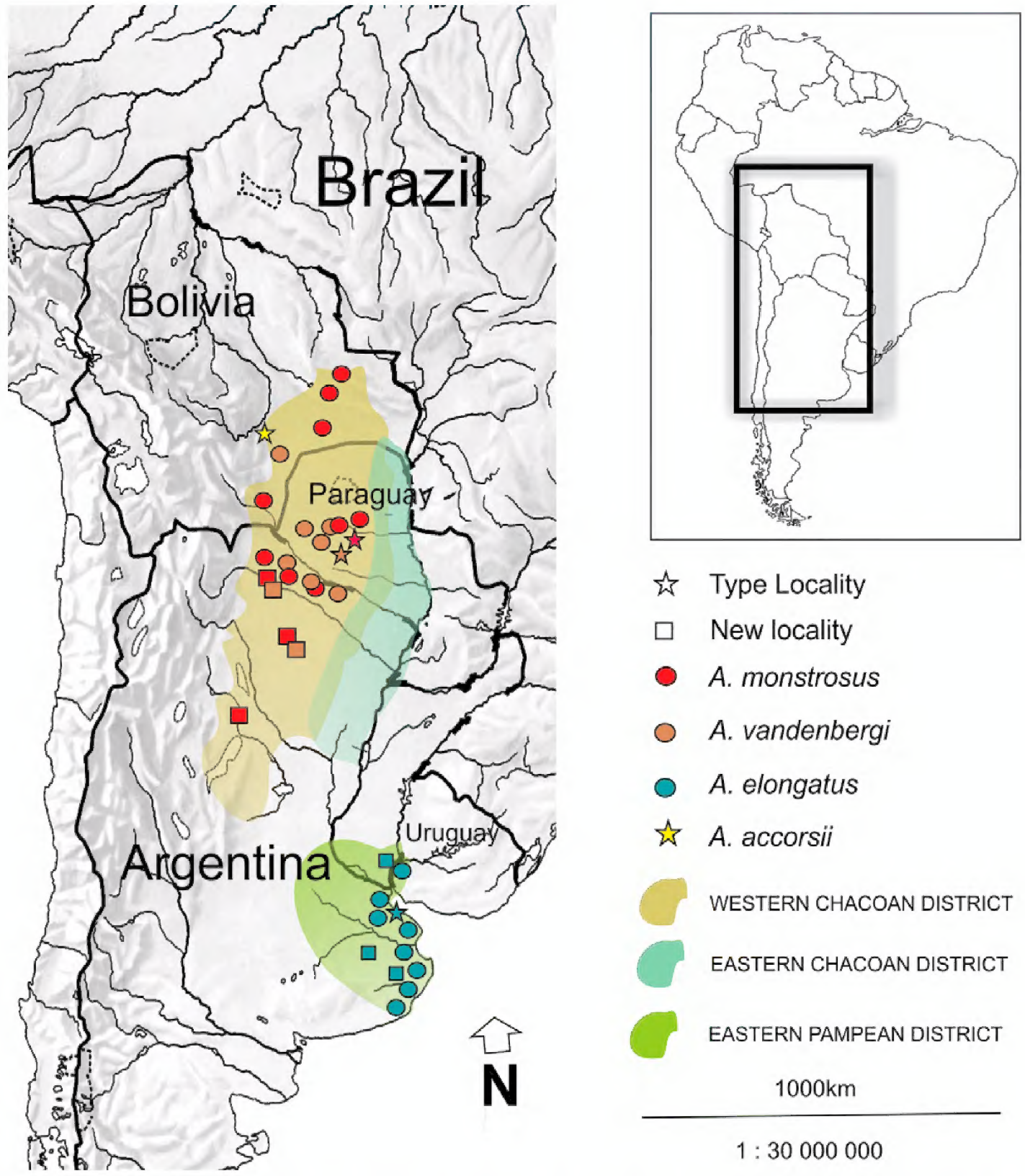

Figure 1. Geographical distribution of *Austrolebias elongatus*, *A. monstrosus*, *A. vandenbergi* and *A. accorsii*. Some Symbols may represent more than one locality.

Félix de Ázara Ichthyological Collection (CFA-IC) was revised.

Based on the databases from herpetological and ichthyological collections of FML (Fundación Miguel Lillo), and the CFA-IC (ex ILPLA: Instituto de Limnología Dr. Raúl A. Ringuelet), a list of all specimens collected by Pierotti and Budín in 1950 was compiled. This list was ordered chronologically, and an itinerary of dates and collecting localities was made. It is noteworthy that Pierotti and Budín and other collectors of FML went by train (taking the railroad "Ramal C25 Ferrocarril Belgrano") on their collecting trips. The FML had a laboratory wagon for this purpose, so they generally used train stations as base camps (G. Scrocchi pers. com.).

Examined material:

***Austrolebias monstrosus*.** CI FML 7097, 2 specimens. Departamento de Rivadavia, Salta province, Argentina (24°08′07.94″S, 062°46′45.47″W), 20 January 2014. CI FML 7098, 2 specimens. General Güemes Camino a Güemes, 10 km northwest of Misión Nueva Pompeya, Chaco. Argentina (24°51′12.2″ S, 061°32′38.2″ W) 12 March 2009. Collectors: Diego Baldo, Claudio Borteiro, Francisco Kolenc, Dardo Andrea Martí and Federico Marangoni. ILPLA 993a, 6 (1 clear & stained), Unknown locality or date, listed as "Corrientes: Ituzaingó, S. Pierotti and D. Budín, 16 November 1950". USNM 176100. Río Dulce, provincia de Santiago del Estero (Col.: T. Marini, 12 June 1933).

***Austrolebias vandenbergi*.** CI FML 7096 71 specimens. Departamento de Rivadavia. Salta province, Argentina: 20 January 2014 (24°08′07.94″S, 062°46′45.47″ W). CI FML 5318.14 specimens, CI FML 5322. 3 specimens. CI FML 5324. 13 specimens, CI FML 5337. 2 specimens., CI FML 5340. 3 specimens. CI FML 5341. 6 specimens. General Güemes, Chaco province, Argentina. Collectors: Baldo, Borteiro, Kolenc and Martí, March 2009. ILPLA 1819 (ex993b), 1 specimen. Unknown locality or date, listed as "Corrientes province: Ituzaingó; S. Pierotti and O. Budín, 16 November 1950".

***Austrolebias nigripinnis*.** MACN 7886, 6 specimens, Ruta 12 km 32, Ituzaingó, Corrientes province. Collector: unknown. 07 August 1987. MACN 6449, Esteros de Cambán, Corrientes. Collector: A. Bachamnn. MACN 8652, 3 males and 5 females, 19.9–24.0 mm SL, Bañados del río Guaviraví, Yapeyú, Corrientes province. Collector: P. Calviño, 2003; MACN 8653, 7 specimens., 27.0–24.5 mm SL, Bañados del río Aguapey, 5 km before Alvear, Corrientes province. Collector: P. Calviño, 2003; MACN 8654, 1 male y 1 female, 28.8–35.8

Figure 2. *Austrolebias monstrosus* in lateral view, anterior to right, two males, from Río Dulce, provincia de Santiago del Estero, Argentina; collected by T. Marini, 12 June 1933 (USNM 176100). Photo: Sandra J. Raredon.

mm LE, Bañados del A° Curuzú Cauatia, R 14 km 436, Corrientes, Collector: P. Calviño, 2003; MACN 9733, 31 ejemplares, Yapeyú, Corrientes. Collector: P. Calviño, 2003.

Austrolebias bellottii. CI FML 341, 1 specimen., Laguna Iberá, San Martín, Corrientes province (28°30′ S, 057°09′ W). Collector: S.A. Pierotti, 9 November 1949. MLP 11081, 30 specimens. (12–24.4 mm LE). Alacantarilla a 100 m del centro de Guardaparques (28°33´24″ S, 57°12´31″ W). Laguna Iberá, Esteros del Iberá, Corrientes. Collectors: J. Casciotta y A. Almirón. 11 April 2005.

Two specimens, from Río Dulce, Santiago del Estero province, Argentina (collector: T. Marini, 12 June 1933), are identified as *Cynolebias elongatus* in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM 176100). Using photographs supplied to us, we re-identify these specimens as *Austrolebias monstrosus* (Figure 2). Additionally, this species was found at 17°42′48″S, 061°08′56″ W (Juriji Phunkner pers. com.), and although specimens were not preserved, a photograph of one specimen is available which allowed us to identify it.

The examined specimens were identified following Costa (2006) and Nielsen and Pillet (2015). *Austrolebias monstrosus* and *A. elongatus* are diagnosed by a combination of characters: transverse series of small scales on anal-fin base in males; contact organs on the outer surface of the pectoral fin and on caudal fin in males; long jaws (lower jaw 29.3–36.4% head length in males); numerous gill-rakers on the first branchial arch (5–6 + 14–16); and minute scales on pectoral-fin and caudal-fin bases in older males. *Austrolebias monstrosus* differs from *A. elongatus* by having gray bars on the flanks in males and fewer dorsal-fin rays in males (16–18 vs. 18–20).

According to Costa (2006), *A. vandenbergi* is diagnosed by a combination of characters including: transverse rows of scales on anal-fin base in males; prominent papillate contact organs on rays of anterior half of anal fin in males; 5 + 14–16 gill-rakers on first branchial arch; urogenital papilla not attached to anal fin; dorsal-fin origin posterior to anal-fin origin in males; flanks light bluish gray to pale golden with narrow faint gray bars in males; anal-fin rays 29–31 in males and 27–29 in females; longitudinal series scales 31–33; and frontal squamation H-patterned.

The itinerary of the Pierotti and Budín expedition was reconstructed as follows: 15 October to 3 November 1950: Ituzaingó, Corrientes province; 6 November: Embarcación, Salta province; 7 November: Ituzaingó, Corrientes province; 9 to 10 November: Laguna Oca, Formosa province; 14 November: Laguna Oca, Formosa province and Aguaray, San Martín department, Salta province. Remarkably, there is an inconsistency, the lot

"ex ILPLA 993", corresponding to *A. monstrosus* and *A. vandenbergi*, supposedly was collected on 16 November 1950 at Ituzaingó, Corrientes province by Pierotti and Budín. However, there are two additional lots with the same collecting date: one lot from Esteros de la Laguna Oca (Formosa province), collected by Pierotti alone, and another from the city of San Miguel de Tucumán (Tucumán province), collected by by Budín. Continuing with the reconstructed trip itinerary we have, after that date, the localities Luna Muerta, San Martín department, Salta province on 18 November and then, from 20–28 November several collecting localities in Formosa province. By comparison of other material collected at the same time by these collectors, we concluded that the specimens of *A. monstrosus* and *A. vandenbergi* said to be collected from Ituzaingó were not in their original jar and without the original labels.

Here, we restrict the distribution of *A. vandenbergi* and *A. monstrosus* to the Bermejo River basin and the Pilcomayo River basin floodplains of the dry Western Chacoan district (*sensu* Morrone 2014). Our reasons are:

1) The lot supposedly from Ituzaingó were not in their original jar and original labels missing, which implies that they probably were transferred to a new jar with a new label.

2) The itinerary of the collecting trip by Pierotti and Budín is inconsistent with the supposed date and locality informed in the lot ILPLA 993 from Ituzaingó. After tracking the collecting localities and dates of the expedition it is unlikely that the collectors would go back to Ituzaingó on 16 November 1950 after having been there for 18 days only two weeks before. The most likely place of collection, if we consider the date as valid, would be somewhere between Esteros de Laguna Oca (Formosa province) and San Martín (Salta province) in the western Chacoan region.

3) Further ecological and biogeographical considerations support that these species are not present in the wet Eastern Chacoan district, near the Paraguay and Paraná rivers. In this area we observed a different composition of seasonal rivulids, together with different environments (Figure 3), ecological conditions and seasonal filling drying cycles compared to those where we recorded *A. vandenbergi* and *A. monstrosus*. Additional indirect evidence supports this statement. Many specimens of *A. vandenbergi* and *A. monstrosus* have been collected by Pierotti and other collectors from the basins of Bermejo and Pilcomayo rivers, in the dry Western Chaco. The specimens supposedly from Ituzaingó are the only known to have been collected in the Paraguay-Paraná floodplain, although this is a very well surveyed area (i.e., Casciotta et al. 2005) and if these species would be present there one would expect additional records in the area. In fact, in this area other species of seasonal killifish are found: *A. bellottii* (Steindachner, 1881) and

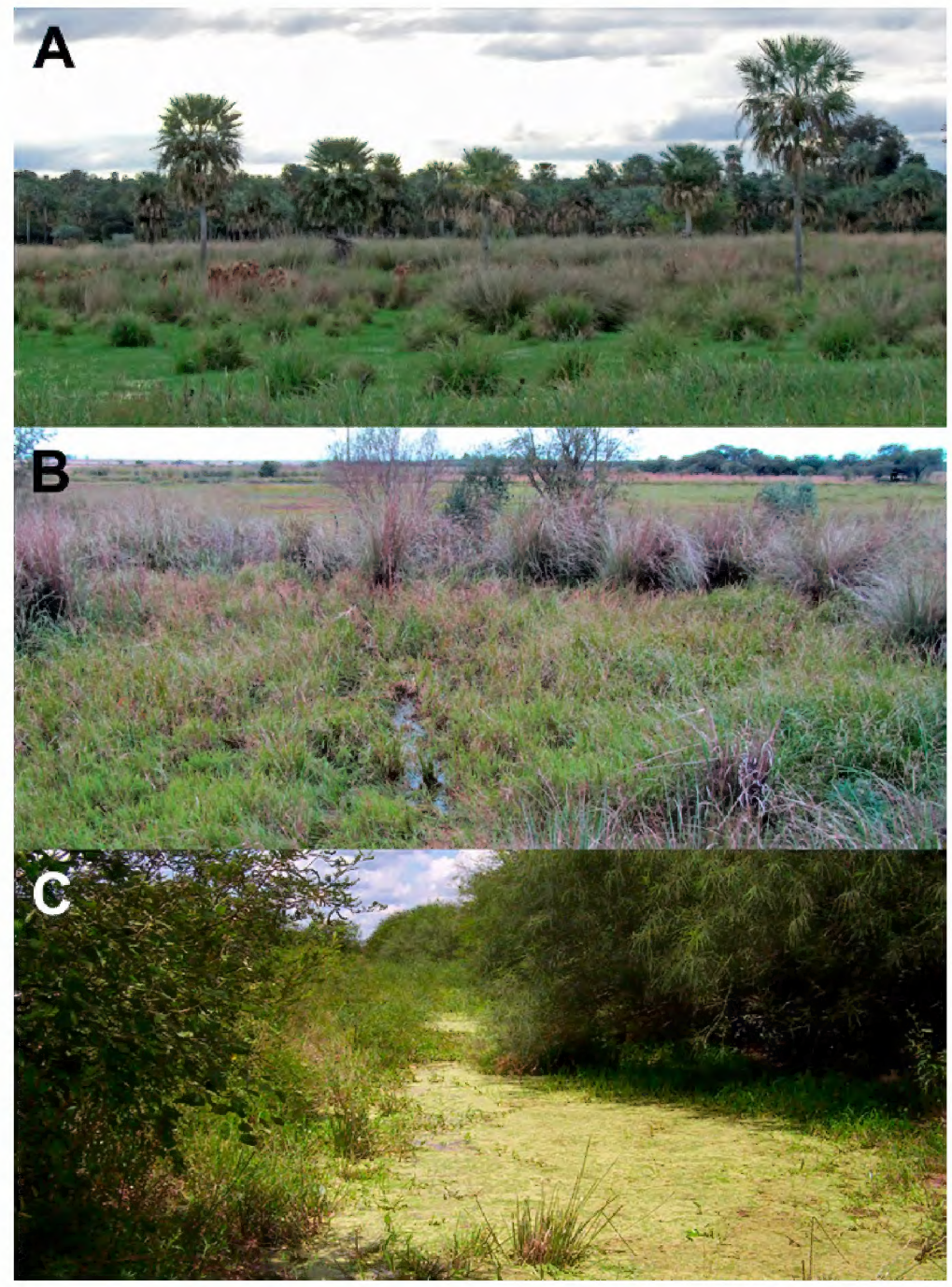

Figure 3. Seasonal ponds from different regions. **A:** Eastern Chacoan district, Río Paraguay basin at Formosa, inhabited by *Austrolebias bellottii*, *A. nigripinnis*, and *Pterolebias longipinnis*. **B:** Corrientes near Ituzaingó, Paraná province (*sensu* Morrone 2014), inhabited by *A. bellottii* and *A. nigripinnis*. **C:** Western Chacoan district in Salta inhabited by *A. monstrosus*, *A. vandenbergi*, *Trigonectes aplocheiloides*, *Neofundulus paraguayensis* and *Papiliolebias bitteri*.

A. nigripinnis (Regan 1912), (pers. obs.). We have intensively sampled for many years within the provinces of Corrientes, Chaco, Formosa and Salta (Figure 4), and have revised all lots of rivulids in the four main ichthyological collections of Argentina (MACN, ILPLA, MLP, CI-FML) but have recorded only *A. monstrosus and A. vandenbergi* in the dry Western Chacoan district. The wet Eastern Chacoan district and Corrientes (Espinal district in the west and Paraná province in the east, *sensu* Morrone (2014) show a very different ecology and species composition not present in the Western Chacoan district. Corrientes lies within the Lower Paraná freshwater ecoregion, but all the known records of *A. monstrosus* and *A. vandenbergi* are in the Chaco freshwater ecoregion (*sensu* Hales and Petry 2015), and particularly in the Western Chacoan district. In this last area, seasonal ponds fill around December and dry out around March (pers. obs.) in accordance with seasonal precipitation (Servicio Meteorológico Nacional 2016). In contrast, the Eastern Chacoan district presents a relatively dry summer, with a bimodal rain in autumn

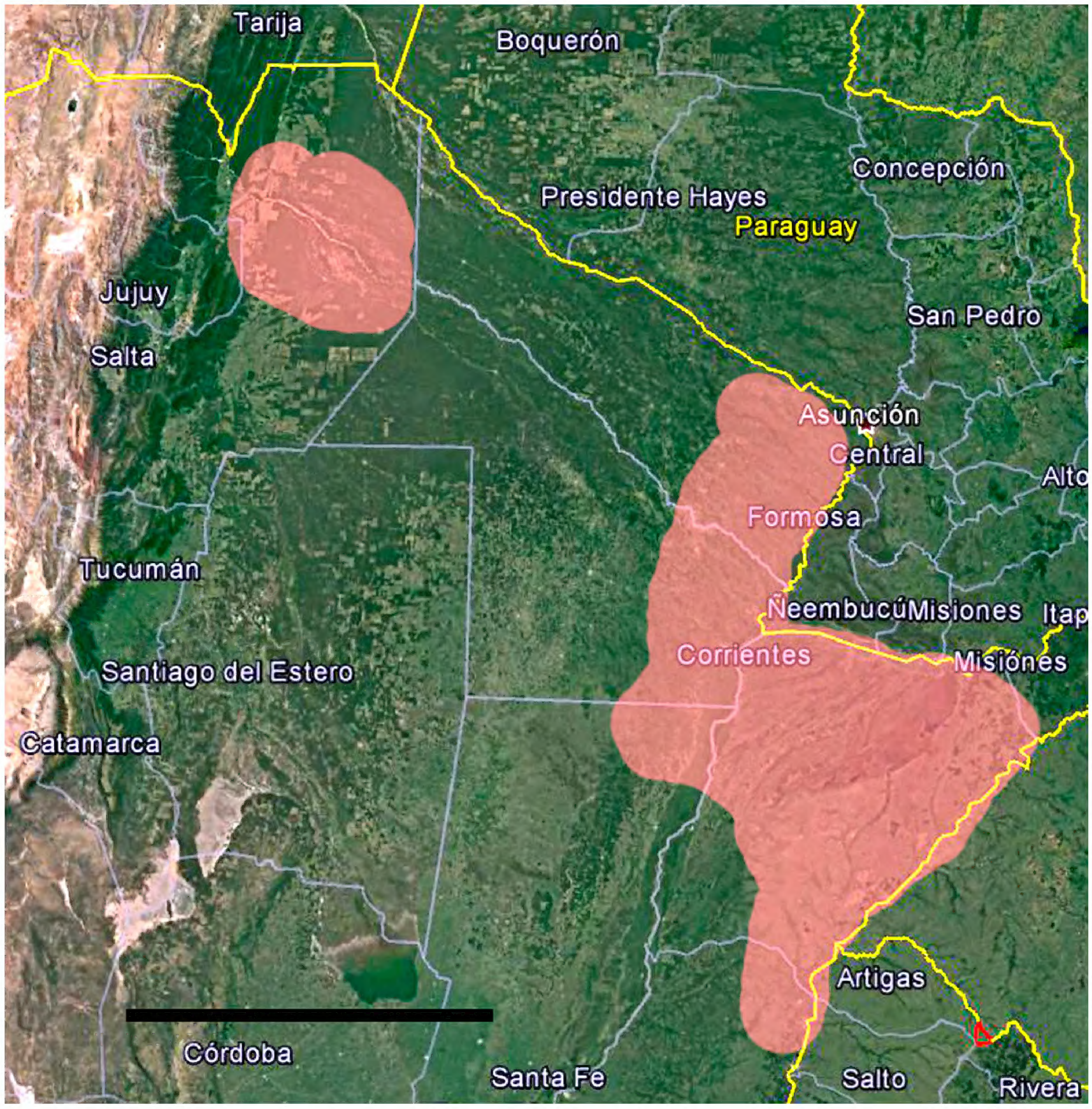

Figure 4. Sampled area highlighted in red. Black bar = 430 km.

and spring and a cold dry winter with higher annual precipitations. This precipitation pattern is the same in the south, in the Pampean region, with the main difference being cooler weather and higher precipitation increasing toward the south (Servicio Meteorológico Nacional Argentino). Ponds in the Eastern Chacoan district tend to fill in autumn and spring and dry in winter and summer. Similarly, in the pampas floodplains near Buenos Aires and Entre Ríos, two filling cycles may be observed in those periods, but higher precipitation and cooler weather in these areas may join the spring and autumn cycles into one cycle with only partial drying in winter (Alonso et al. pers. obs.). This determines a relative ecological continuum between the Paraguay and Paraná floodplain with species such as *Austrolebias nigripinnis* and *A. bellottii* inhabiting almost all this area. Therefore, an ecological restriction for the distribution of seasonal killifish across very different pond cycles and weather is present between Western and Eastern Chacoan districts, although no geographical barriers seem to limit the distribution of fish in this area. Excluding Ituzaingó for *A. vandenbergi* and *A. monstrosus* implies a reduction in around 585 linear kilometres of their distributional range and also implies that these species are not present in the Paraguay–Paraná basin nor in the Eastern Chacoan district and restrict their distribution to the Western dry Chaco.

Nielsen and Pillet (2015) stated that the records of *A. monstrosus* (Osinaga 2006) and *A. vandenbergi* (Montaña et al. 2012) from Bolivia correspond to *Austrolebias accorsii,* but they did not revise the material. Instead, they assumed it to be *A. accorsii* because it was collected in the Río Parapetí basin, near the Río Grande basin from where *A. accorsii* is only known at its type locality (Figure 1). The specimen figured by Osinaga (2006) is clearly *A. monstrosus*. We consider Osinaga (2006) as a valid record for *A. monstrosus*. This species was also collected by Juriji Phunkner in the Río Grande basin (pers. com.), who also

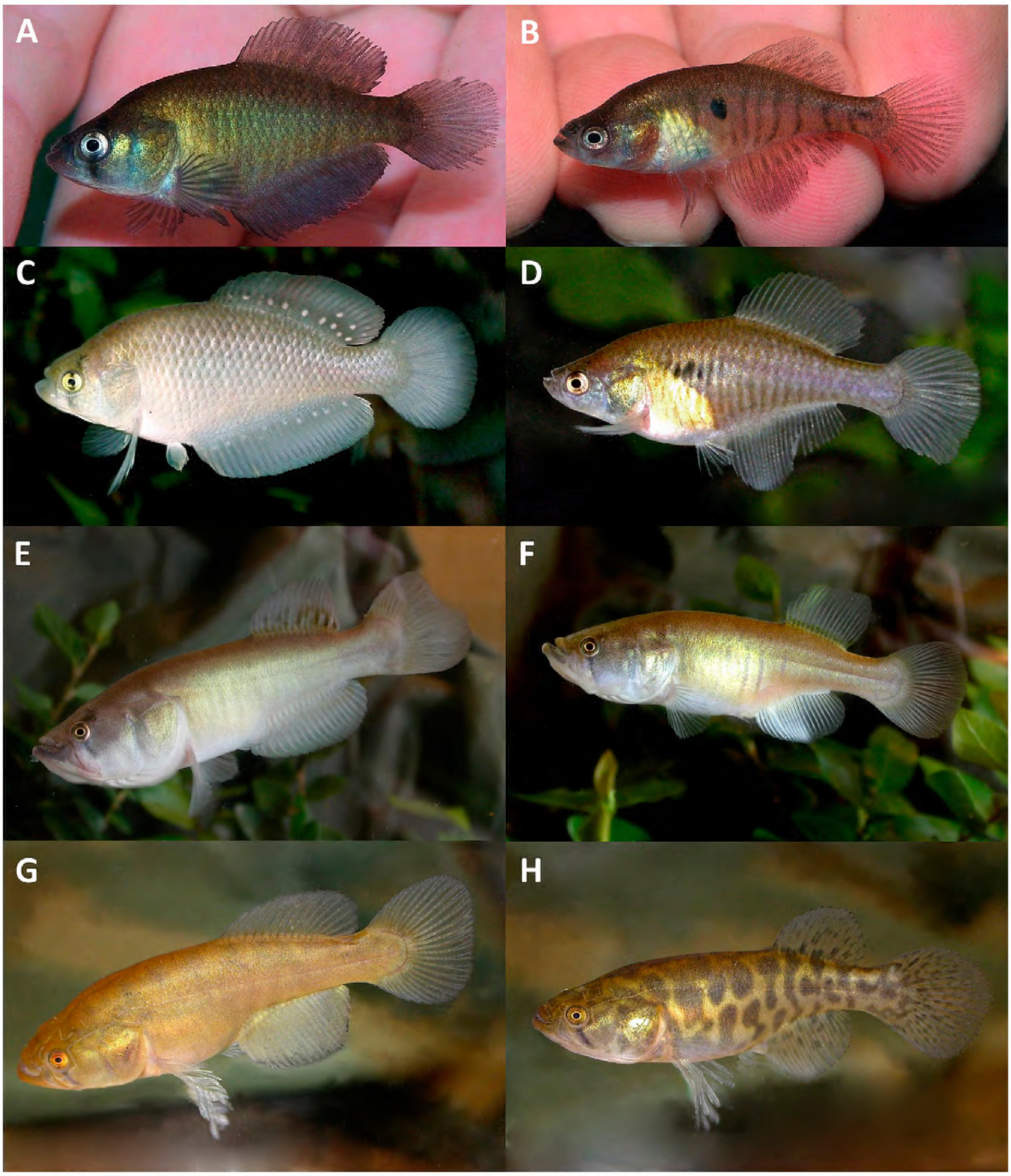

Figure 5. Species treated in the present study. **A, B:** *Austrolebias accorsii* F1, from type locality. **C, D:** *Austrolebias vandenbergi* from Bermejo river basin at Salta province, Argentina. **E, F:** *Austrolebias monstrosus* from Bermejo River basin at Salta province, Argentina. **G, H:** *Austrolebias elongatus* from Azul, Buenos Aires province. **A, C, E, G:** males. **B, D, F, H:** Females. Photos: Juriji Phunkner (A, B); Pablo Calviño (C–G).

collected what appears to represent the northernmost record for *A. monstrosus* (75 km north of the closest previous locality). Although specimens were not preserved, a photograph of one specimen collected by Phunkner was studied. We consider that the specimens figured by Montaña et al. (2012) are *A. vandenbergi* because they do not match the diagnosis of *A. accorssii* but do match *A. vandenbergi* (*sensu* Costa 2006).

Remarkably *A. accorsii* was described based on old specimens kept in aquarium (Guillaume Dethu pers. com.) and actually present a body shape not representative for the species. Juriji Phunkner kindly provided us with photographs of F1 aquarium specimens of this species from the type locality (Figure 5A). Interestingly these specimens present vertical bars also seen in the figure of the description of this species. Vertical bars

are lost in old individuals of *A. vandenbergi* (Figure 5C). The specimen in Figure 5A and the holotype figured in the original description of *A. accorsii* present transverse rows of scales on anal fin base. These two diagnostic characters should be reconsidered. Given that the type series of this species is based on old specimens the linear measurements should be used with caution. A redescription of this species using fresh material is needed. Juriji Phunkner kept these species in an aquarium and stated that *A. accorsii* is smaller than *A. vandenbergi*, which he collected in the same area.

Distribution of rivulids in the Chaco and Pampasic floodplains is probably shaped by ecological constraints rather than to geographical barriers because this flat region is commonly flooded, facilitating dispersion events even between basins (pers. obs.). This explains why fauna typical of the Paraguay–Paraná river basins are also found in nearby basins within the same ecological region, such as the Mamoré and Amazon basins (Nielsen and Pillet 2015). The lack of barriers in this region allow these rivulids to have wide distributions in contrast to what is seen elsewhere (Costa 2010). Consequently, these species have large populations with great intraspecific variability and geographical variations (pers. obs.; Fromm 1996a, 1996b) that should be carefully considered when evaluating their taxonomy. The Province of the Great Rivers according to López et al. (2008), is more defined by the connectivity of river basins than with ecology. In contrast, in seasonal fishes of the chaco-pampean floodplain the ecological constrains of the seasonal pond's filling/drying cycles may be equally relevant although distribution of species is directly related to permanent water bodies (pers. obs.; Costa 2010).

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